THE REARING OF SERPULID LARVÆ WITH NOTES ON THE BEHAVIOR OF THE YOUNG ANIMALS.[1]

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The Method of Rearing.

The complete success of an attempt to rear serpulid larvæ[2] has prompted the present description of the method, in view of the fact that other workers have been unsuccessful in the same field.[3] It is hoped that the method may be found valuable not only for serpulid larvæ, but also for the great majority of forms which do not allow a direct current of water to be passed in and out of the dish containing them.

The eggs after fertilization were placed in large glass beakers and the water was changed several times during the first day. They were then allowed to develop into free swimming trochophores which collected in great numbers at the surface of the water during the second day. A few of these were removed with a pipette and placed in a "battery jar" containing fresh sea water obtained in the open harbor at rising tide on a bright afternoon. Not more than a few hundred larvæ were put into any one battery jar. The jars were covered with glass plates to keep out the dust, and were placed in such a position that the rising sun shone on them for about an hour just after sunrise each morning. Immediately after the sun bath they were cooled off in buckets of cold spring water and were then placed for the rest of the day in shallow basins of the same cold water. In this way the proper conditions for the development of the microscopic algæ and other organisms which serve as the food of the larvæ

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 65.

[2] *Hydroides dianthus.*

[3] The work described was done at the Cold Spring Harbor Biological Station during the summer of 1902, while the writer was holding the John D. Jones Scholarship at that place. My best thanks are due to Professor C. B. Davenport, the director of the station, for his kindness in supplying me with every possible facility while at the laboratory. Some of the notes refer to work done at the Naples Zoölogical Station during the winter of 1902–3.

were furnished without at the same time heating the water to such an extent as to kill the larvæ themselves.

Under the above treatment the larvæ flourished and successfully went through the transformation from the free-swimming to the sedentary condition. They settled down on the sides of the vessels in great numbers about fourteen days after fertilization and observations could readily be made upon them with a horizontal microscope. The examination of the larvæ can undoubtedly be facilitated by the suspension of glass slides in the water at the places where the free-swimming larvæ are about to attach themselves. The slides with the attached larvæ can then be transferred to shallow dishes of sea water and examined with the microscope in its ordinary upright position.

A study of the development of the opercula in the serpulids constituted my main object in rearing the larvæ and a description of this feature is incorporated in a paper now in press.[1] A few incidental observations on the behavior of the young animals are given in the following notes :

Behavior of the Young Serpulids.[2]

The Free Swimming Larvæ. — As soon as the pre-oral band of cilia is well developed the young larvæ swim toward the surface of the water and collect there in great numbers, especially at the edges near the glass sides of the jar. They are always more crowded in certain regions than in others, but the exact relation of this crowding to a phototactic response was not made out because of the complex relations due to refraction and reflection of the light within the jar. The greatest crowding was usually on the side of the jar facing the window and on the side directly away from the window. At the latter place the collection of larvæ may have been due to a secondary reflection of the light within the jar.[3]

[1] "Compensatory Regulation." To appear in the *Journal of Experimental Zoölogy*, Vol. II., No. 1.

[2] *Hydroides dianthus* (at Cold Spring Harbor), unless otherwise noted.

[3] A. Giard ('76) in his "Note sur l'embryogénie de la Salmacina Dysteri Huxl." (*Compt. Rend.*, Tome 82, 1876, pp. 233–235, 285–288) says that the free-swimming larvæ of *Salmacina* collect on the sides of the aquarium facing the light while they settle down and form tubes on the side away from the light.

Attachment of the Larvæ. — The period at which attachment of the larvæ should take place is a critical one and death is the usual result under ordinary laboratory conditions. In my experiments at Cold Spring Harbor, however, there were hundreds of survivors in each jar. On account of the frequent change of position of the jars, as well as the complex refractions and reflections within them, I was not able to find out whether the place of attachment of the larvæ has any definite relation to the source of the light. The young Serpulids were found to be fairly uniformly distributed, though there were groupings at several different places. One of these consisted of a large number collected on the glass just below the surface film of the water. These formed a band surrounding the jar, but it is interesting to note that they did not grow as rapidly as those lower down, although fresh sea water was added in each case to raise the level slightly and make up for evaporation. Going down from this band-like zone of greatest frequency the number of individuals decreased until the bottom of the vessel was reached, where again there was a considerable number, especially in the corner between the bottom and the sides of the jar.

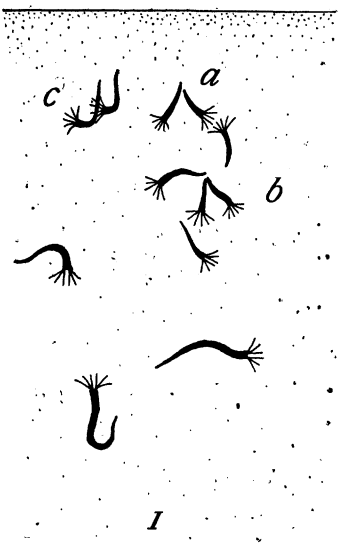

FIG. 1. (× 4.) A group of the young of *Hydroides dianthus* as attached to the side of a battery jar. The level of the water surface is shown. *a* and *b* are groups showing radial divergence of the tubes. *c* shows two tubes which have evidently responded to a lateral stimulus. No definite relation to gravity is shown.

Tube Formation. — The tube when first formed is a very narrow, almost transparent ring of calcareous matter, the body of the short larva extending out of it at both ends. This ring is secreted by the region just back of the free anterior end of the thoracic membrane and as its formation goes on the animal can be seen to extend its thoracic membrane over the anterior edge of the tube in order apparently to smooth the edges and get the

material in shape to fit the body. At such a time the body may project a considerable distance from the anterior end of the tube. The tube is deposited quite rapidly. In the case shown in Fig. 2 the amount of growth in the course of twenty hours is given. This is equal to .29 mm. or .35 mm. per day.

I tried to discover some regularity in the arrangement of the axes of the tubes with respect to gravity, light and food conditions but was able to find no general rule in the matter although some groups seem to be arranged with respect either to maximum food-obtaining ability or with respect to a lateral stimulus of unknown character. Fig. 1 which is reproduced from my notebook gives a small section of a side of one of the jars. Evidently there is no general rule of arrangement though radial divergence of the kind shown at *a* and *b* (Fig. 1) may be explained on a utilitarian basis as a spreading out from a central point in order to obtain more feeding room. The arrangement at *c* however does not come under this head but must be considered as a very definite response to a lateral stimulus. It is hard to conjecture what this stimulus may have been.

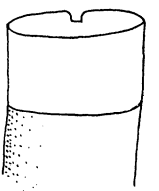

FIG. 2. (× 38.) Open end of a tube of *Hydroides uncinata*. The unshaded portion represents the amount of addition to the tube in the course of twenty hours.

General Activities of the Young at a Later Stage. — The following observations were made on young individuals during the few days preceding and the few days following the formation of the operculum. Specimens of *Hydroides uncinata* and *H. pectinata* observed at the Naples Zoölogical Station during the winter of 1902–1903 as well as the *H. dianthus* of Cold Spring Harbor were used.

The two eye spots are very prominent at this stage and a sudden shadow such as is produced by the passing of a hand between the animals and the source of light causes the serpulids to contract and withdraw with a jerk into their tubes. The branchiæ are then completely hidden inside of the tube and before the development of the operculum their ends form a barricade a short distance within the opening. After a short period of inaction if there is no new disturbance the fine thread-like

ends of the branchiæ begin to appear and wave back and forth around the mouth of the tube as if feeling for signs of danger. In this position the animal usually remains for several minutes often with nothing more than the ends of the two longest branchiæ projecting from the tube. After satisfying itself in this manner the animal pushes itself forward and expands the whole branchial crown with comparative rapidity. After the development of the functional operculum this organ is pushed to one side and the branchial ends are slightly projected around its margin before the thrusting out of the whole branchial circlet takes place (see Fig. 3). The young animals, as well as the adults, are extremely sensitive to the slightest mechanical jar, a very small shock being sufficient to keep them within their tubes for a considerable time. At Naples I was often greatly annoyed while attempting to make drawings of the animals in an extended state to see them jerk back into their tubes at the sound of a band starting to play in the adjacent park. Under such conditions they "sulked" in their tubes until the musical selection was finished.

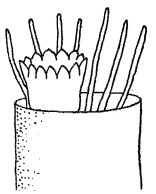

FIG. 3. (× 38.) *Hydroides pectinata*. The young serpulid has thrust out the ends of its branchiæ preparatory to the expansion of the whole branchial circlet.

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